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Research was conducted to test the hypothesis that activation of the calcium-dependent protease, calpain, is involved in the induction of long-term potentiation (LTP) of synaptic transmission and memory storage in the mammalian brain. Evidence indicates that naturally-occurring patterns of synaptic activity can induce LTP by activating an NMDA receptor that allows postsynaptic influx of calcium. Activation of NMDA receptors induces a calcium-dependent proteolysis of spectrin, a calpain substrate; both calpain and spectrin are present in dendritic spines. Both a calpain inhibitor and an NMDA receptor antagonist have been found to interfere with spatial and olfactory learning. Calpain-mediated spectrin degradation occurs in vivo after various treatments; studies of simple cell types suggest that this mechanism may produce structural changes similar to those which accompany LTP.

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The purpose of the research proposed in this grant is to test the hypothesis that a specific biochemical mechanism is responsible for storage of memories for "facts" or "data" (as opposed to "procedures", rules", or "habits") in the mammalian brain. We proposed that the encoding process involves: 1) an unusual pattern of physiological activity in the relevant neural pathways, 2) influx of calcium into dendritic spines postsynaptic to the active axons, 3) activation of the calcium-sensitive protease calpain, 4) partial degradation of spectrin - a protein that regulates membrane surface chemistry and possibly spine shape, and 5) a rearrangement of postsynaptic structure that results in a stable potentiation of postsynaptic potentials (long-term potentiation or LTP).

To date, progress has been made in the following areas:

1) Activity patterns and LTP induction.

We have demonstrated that axonal stimulation patterns incorporating two naturally-occurring aspects of hippocampal physiology - burst firing and the theta rhythm - are particularly effective for LTP induction (Larson, et al., 1986). A single stimulation burst does not induce LTP but "primes" the postsynaptic cells such that EPSPs evoked by a subsequent burst are prolonged and exhibit enhanced temporal summation (Larson and Lynch, 1986). This priming effect is due to a suppression of IPSPs that is maximal 200 msec (the theta period) after the priming burst (Larson and Lynch, submitted); the enhanced response to a primed burst allows it to trigger N-methyl-D-aspartate (NMDA) receptor-mediated currents which are essential for LTP induction (Larson and Lynch, 1988). Work in other laboratories indicates that NMDA receptors are linked to calcium channels; earlier work from our laboratory indicated that a postsynaptic influx of calcium is the trigger for LTP (Lynch, et al., Nature 305:719-721, 1983).

2) Subcellular localization of calpain and spectrin.

The hypothesis requires that both calpain and spectrin be present in dendritic spines. Using electron microscopy, we have confirmed that antibodies to calpain do stain spines and dendrites (Perlmutter, et al., 1988). Furthermore, subcellular fractionation experiments have shown that calpain activity is highest in soluble form in synaptosomal fractions (Baudry, et al., 1987). Finally, we have shown that contrary to a prior report (Reiderer, et al., J. Cell. Biol. 102:2088-2097, 1987), antibodies that recognize both spectrin and its calpain-mediated breakdown product (see below) do stain dendrites and spines in brain (Ivy, et al., in press).

3) Physiological restrictions on possible LTP substrates.

We have conducted experiments studying interactions between extracellular calcium concentrations, paired-pulse facilitation, and LTP to limit the possible mechanisms for the expression (substrate) of LTP. Paired-pulse facilitation in hippocampus appears to be identical to facilitation at neuromuscular junction where considerable evidence indicates it to be due to enhanced presynaptic calcium currents (Muller and Lynch, submitted). Induction of LTP has no effect on facilitation, suggesting that LTP is not due to an enhancement of presynaptic calcium currents (Muller and Lynch, submitted). Further, when extracellular calcium is raised to the point where single-pulse responses are asymptotic, facilitation is still observed. This suggests that synaptic transmission in hippocampus is not limited by postsynaptic receptors and hence, that LTP is not likely to be due to a simple increase in number of postsynaptic receptors (Muller and Lynch, submitted). Finally, when the extracellular magnesium concentration is low (10-20 uM), an NMDA receptor-mediated component of single EPSPs can be measured. The proportion of this component of the response remains constant during facilitation, but is decreased during

LTP (Muller and Lynch, submitted). This suggests that LTP cannot be due to a simple increase in transmitter release.

4) Spectrin proteolysis by calpain in vivo.

Proteolysis of spectrin by calpain produces a 150 kDa breakdown product (BDP) that is recognized by spectrin antibodies. We have refined an immunoblot assay of the spectrin BDP and can detect nanogram quantities of the peptide. The BDP only appears when spectrin and calpain are incubated with calcium; production of the BDP is prevented by leupeptin (a calpain inhibitor) and accelerated by calmodulin (Seubert, et al., 1987). We have used this BDP assay to test for calpain activation in vivo by various treatments: a) Lesions of the entorhinal inputs to the dentate gyrus result in the degradation of 20-25% of the spectrin present in dentate, most of which appears as the calpain-mediated BDP. The increase in BDP appears within four hours after lesion, is maximal two days later, remains present for at least four weeks, and is largely suppressed by chronic intraventricular infusion of leupeptin (Seubert, et al., in press). b) Injections of colchicine into hippocampus results in cell death; this pathological response is preceded by an increase in the spectrin BDP (Seubert, et al., submitted). c) In brindled mice (genetic deficiency in copper metabolism), an increase in spectrin BDP appears throughout the forebrain at about postnatal day 12. This BDP increase can be prevented by copper treatment which also prevents the widespread neuropathology that precedes death in these animals (Seubert, et al., in prep.). d) Treatment of rats with trimethyl tin results in selective cell death in the CA1 field of hippocampus. This treatment is also accompanied by a selective increase in the calpain-mediated spectrin BDP in CA1 (Turnbull, et al., in prep.).

5) Shape change induced by calpain-spectrin interactions.

The hypothesis requires that the calpain-spectrin interaction produce the structural changes that we and others have observed to accompany LTP. While partial cleavage of the cytoskeleton underlying the membrane would a priori be expected to produce morphological reorganization, we have experimentally explored this point using two types of simple cells: blood platelets and erythrocytes. Platelets undergo a shift from a flattened, smooth configuration to a globular shape with many protrusions upon treatment with certain hormones; using immunoblots, we found that spectrin is degraded during shape change resulting in the appearance of the same BDP produced by calpain (Kramer, et al., submitted). Comparable results were obtained using erythrocyte shape change as the test system (Siman, et al., 1987). Clearly, activation of the calpain-spectrin interaction is capable of producing the types of anatomical changes found after LTP induction.

Related to the problem of the biochemical mechanism of LTP induction, we have also examined the role of protein kinase C. Others laboratories have reported that phorbol esters that activate kinase C produce an LTP-like form of synaptic potentiation. However, we have found that the EPSP facilitation induced by phorbol esters does, in fact, wash out with the drugs (Muller, et al., in press). That washout of phorbol esters requires prolonged periods in slices probably explains the discrepancy in results. Furthermore, we have found that the reported difficulty in inducing LTP by electrical stimulation in the presence of phorbol esters probably is due to the drugs' reducing the postsynaptic responses to high frequency stimulation (Muller, et al., in press).

6) Activation of calpain in hippocampal slices by NMDA.

The critical role of NMDA receptors in LTP induction led us to test for spectrin degradation in hippocampal slices exposed to NMDA receptor agonists. Bath application of NMDA produced a profound depolarization of neurons in the slice. Subsequent immunoblot assay of spectrin demonstrated that NMDA treatment greatly accelerated the production of the calpain-mediated spectrin BDP. Both the neuronal depolarization and spectrin breakdown were prevented by treatment with an NMDA receptor antagonist. Neither NMDA treatment in the absence of extracellular calcium nor depolarization induced by KCl resulted in spectrin breakdown (Seubert, et al., submitted).

7) LTP and memory: pharmacological correlates.

We had previously shown that chronic intraventricular infusion of leupeptin (a calpain inhibitor) disrupts memory storage in both a spatial maze and an olfactory discrimination task (Staubli et al., Behav. Neur. Biol. 40:58-69, 1984; Brain Res. 337:333-336, 1985). We have now shown that this treatment also reversibly inhibits the induction of LTP (Staubli, et al.,1988). We have also tested the effects of chronic infusion of the NMDA receptor antagonist AP5 (which is known to prevent LTP induction - Morris, et al., Nature 319:774-776, 1986) on olfactory learning. We found that AP5 prevented the acquisition of new olfactory discriminations but had no effect on memory for already-learned discriminations (Staubli, et al., in press).

In summary, work over the past two years has: 1) identified naturally occurring activity patterns that produce LTP and the physiological events involved, 2) somewhat restricted the possible substrates for LTP, 3) localized both calpain and spectrin to postsynaptic sites, 4) demonstrated that spectrin degradation most likely produced by calpain can occur in vivo, 5) shown that shape changes of the type observed during LTP can be mediated by the calpain-spectrin interaction, 6) demonstrated that activation of NMDA receptors can trigger calcium-dependent proteolysis of spectrin, and 7) shown that two treatments that interfere with LTP also produce profound impairments in memory formation.

PUBLICATIONS

Baudry, M., DuBrin, R. and Lynch, G. Subcellular compartmentalization of calcium-dependent and calcium-independent neutral proteases in brain. Synapse, 1987, 1,506-511.

Ivy, G.O., Seubert, P., Baudry, M., and Lynch, G. Presence of brain spectrin in dendrites of mammalian brain: Technical factors involved in immunochemical detection. Synapse, in press.

Kramer, K., Seubert, P., Lynch, G. and Baudry, M. Activation of platelets produces a calpain-mediated degradation of a brain spectrin-like protein. Submitted.

Larson, J. and Lynch, G. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. Science, 1986, 232, 985-988.

Larson, J. and Lynch, G. Postsynaptic events controlling induction of long-term potentiation by patterned stimulation. Submitted.

Larson, J. and Lynch, G. Role of N-Methyl-D-Aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal theta rhythm. Brain Res., 1988, 441, 111-118.

Larson, J., Wong, D., and Lynch, G. Patterned stimulation at the theta frequency is optimal for induction of hippocampal long-term potentiation. Brain Res., 1986, 368, 347-350.

Muller, D. and Lynch, G. Calcium dependence of synaptic transmission and plasticity in hippocampus. I. Transmitter release and paired-pulse facilitation. Submitted.

Muller, D. and Lynch, G. Calcium dependence of synaptic transmission and plasticity in hippocampus. II. Long-term potentiation. Submitted.

Muller, D. and Lynch, G. Long-term potentiation differentially affects two components of synaptic responses in hippocampus. Submitted.

Muller, D., Turnbull, J., Baudry, M., and Lynch, G. Phorbol-ester induced synaptic facilitation is different than long-term potentiation. Proc. Natl. Acad. Sci. (USA), in press.

Perlmutter, L.S., Siman, R., Gall, C., Seubert, P., Baudry, M. and Lynch, G. The ultrastructural localization of calcium-activated protease "calpain" in rat brain. Synapse, 1987, 2, 79-88.

Seubert, P., Baudry, M., Dudek, S. and Lynch, G. Calmodulin stimulates the degradation of brain spectrin by calpain. Synapse, 1987, 1, 20-24.

Seubert, P., Nakagawa, Y., Vanderklish, P., Lynch, G., and Baudry, M. Intrahippocampal colchicine injection results in spectrin proteolysis. Submitted.

Seubert, P., Petersen, C., Vanderklish, P., Baudry, M., and Lynch, G. Spectrin proteolysis is increased in brindled mice. In Preparation.

Seubert, P., Larson, J., Oliver, M., Jung, M.W., Baudry, M., and Lynch, G. Stimulation of NMDA receptors induces proteolysis of spectrin in hippocampus. Submitted.

Seubert, P., Ivy, G., Larson, J., Lee, J., Shahi, K., Baudry, M., and Lynch, G. Entorhinal lesions produce a calpain-mediated degradation of brain spectrin in dentate gyrus. I. Biochemical studies. Brain Res., in press.

Siman, R., Baudry, M. and Lynch, G. Calcium activated proteases as possible mediators of synaptic plasticity. In: Synaptic Function (G.M. Edelman, W.E. Gall, and W.M. Cowan, Eds.), John Wiley, New York, 1987, 519-548.

Staubli, U. and Lynch, G. Stable hippocampal long-term potentiation elicited by "theta" pattern stimulation. Brain Res., 1987, 435, 227-234.

Staubli, U., Thibault, O., DiLorenzo, M., and Lynch, G. Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. Behav. Neurosci., in press.

Staubli, U., Larson, J., Baudry, M., Thibault, O. and Lynch, G. Chronic administration of a thiol-protease inhibitor blocks long-term potentiation of synaptic responses. Brain Res., 1988, 444, 153-158.

Turnbull, J., Seubert, P., Lynch, G., and Baudry, M. Effects of trimethyl tin treatment on spectrin proteolysis in rat brain. In preparation.

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